IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: HO *et al.*	Confirmation No.: 4326
Application No.: 09/960,244	Art Unit: 1651
Filed: September 21, 2001	Examiner: Lankford Jr., Leon B.
For: **Cell Populations Which Co-Express CD49c and CD90**	Atty. Docket: 2560.0020000/JAG/DOS

Reply Brief Under 37 C.F.R. § 41.41

Mail Stop Appeal Brief - Patents

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

The present reply is submitted in response to the Examiner's Answer mailed July 9, 2010 (hereinafter the "Answer").

Appellants filed an Amended Brief on Appeal Under 37 C.F.R. § 41.37 (hereinafter the "Appeal Brief") to the Board of Patent Appeals and Interferences for the above-captioned application on January 25, 2010. The appeal is directed to a final rejection of claims 14, 21, 25, 26 & 97. The currently issued Examiner's Answer has withdrawn the rejection of claim 97 under 35 U.S.C. § 112, 1st paragraph, but maintains the rejection of claims 14, 21, 25, 26 & 97 under 35 U.S.C. § 102(b). This is the only rejection remaining in this Appeal. The Answer did not designate any new grounds of rejection. 37 C.F.R. § 41.39(b). The Examiner's Answer includes some new remarks and reiterates many of the erroneous allegations previously asserted in this application. Therefore, in reply to the Answer, Appellants submit this Reply Brief pursuant to 37 C.F.R. § 41.41. Appellants note that all Exhibits cited herein have been previously provided and cited in these proceedings. Exhibit numbers referenced herein are the same as referenced in the pending Appeal Brief.

I. Real Party In Interest

The real party in interest in this appeal is Garnet BioTherapeutics Inc. ("Garnet")(formerly Neuronyx, Inc.), 1 Great Valley Parkway, Suite 12, Malvern, PA 19335, the assignees of record.

II. Related Appeals and Interferences

On April 2, 2010, Appellants filed an appeal in related U.S. Patent Application No. 11/054,824, which is a divisional of the subject application in this appeal. On April 9, 2010, Appellants filed an appeal in U.S. Patent Application No. 10/251,685, which discloses and claims related subject matter, and which is assigned to the real party in interest in the present appeal. Both of these appeals are also currently pending.

III. Status of Claims

Claims 14, 21, 25, 26 and 97 are pending, and claims 1-13, 15-20, 22-24, and 27-96 have been canceled.

Claims 14, 21, 25, 26 and 97 are rejected.

Claims 14, 21, 25, 26 and 97 are appealed.

IV. Grounds of Rejection to be Reviewed on Appeal

The sole remaining ground of rejection alleged against appealed claims 14, 21, 25, 26 and 97 is inherent anticipation under 35 U.S.C. § 102(b). *See*, Answer, pages 3-12.

V. Examiner's Answer and Appellants' Reply

In the Answer of July 9, 2010, the Examiner maintains the rejection of claims 14, 21, 25, 26 and 97 under 35 U.S.C. § 102(b) as allegedly inherently anticipated by Haynesworth *et al.* (U.S. Patent 5,733,542 (1998)) in combination with Pittenger *et al.* (*Science* 284: 143-147 (1999)), Woodbury *et al.* (*Journal of Neuroscience Research* 61:364-370 (2000)), and Lee *et al.* (Hepatology 40: 1275-1284 (2004)). *See*, Answer, page 5, 1st and 2nd paragraphs.

A. A Prima Facie Case of Inherent Anticipation Has Not Been Established.

As more fully set forth in the Appeal Brief, Appellants reiterate that the Examiner has not established a *prima facie* case of inherent anticipation. *See*, Appeal Brief, pages 13-

15, § VII (B) ("Legal Background") and pages 18-20, § VII (C)(2). In particular, Appellants note that inherent anticipation requires that the "missing descriptive material is 'necessarily present,' not merely probably or possibly present, in the prior art." *Trintec Indus., Inc. v. Top-U.S.A. Corp.*, 295 F.3d 1292, 1295 (Fed. Cir. 2002); *see also, Electro Medical Sys., S.A. v. Cooper Life Sciences, Inc.*, 34 F.3d 1048, 1052 (Fed. Cir. 1994). Thus, the judicially created doctrine of inherent anticipation requires that a rejection based on inherent anticipation *cannot be based on mere possibilities or probabilities*; the missing matter must be *necessarily* present in the prior art.

The M.P.E.P. also provides instructions on the requisite burden of proof that an Examiner must provide in asserting a rejection based on inherent anticipation. In particular, the M.P.E.P. states:

> **Requirements of Rejection Based on Inherency; Burden of Proof**
> IV. EXAMINER MUST PROVIDE RATIONALE OR EVIDENCE TENDING TO SHOW INHERENCY
>
> The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic... "*To establish inherency, the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference*, and that it would be so recognized by persons of ordinary skill. *Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient*..." In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art.

See, M.P.E.P. § 2112, IV (emphasis added; internal citations omitted).

Appellants respectfully reiterate that the pending rejection of claims under 35 U.S.C. §102(b) is legally in error and should be reversed because the Examiner has not established a *prima facie* case of anticipation. In particular, the Examiner provides no basis in fact and/or technical reasoning to reasonably support a determination that each and every element of the claimed invention is *necessarily present* in the prior art. This is the case with respect to the independent claims 14 & 97 and also with respect to dependent claims 21, 25 & 26 (the further parameters of which the Examiner has not even addressed with respect to the pending rejection under 35 U.S.C. § 102(b)).

Indeed, despite Appellants' submission of manifold evidence to the contrary, the Examiner maintains a rejection of the pending claims premised solely on subjective

conjecture that the elaimed cell populations "would appear" to be "a population of MSCs as disclosed by Haynesworth." Hence, the pending rejection of claims should be reversed because mere possibility or probability based on conjecture does not meet the requisite standard for asserting an inherent anticipation rejection. *See e.g.*, Answer, page 8, 2nd paragraph; *see also*, discussion below with respect to Examiner's continued reliance on the unsupported preamble "it would appear." Accordingly, Appellants respectfully request that the rejection of claims 14, 21, 25, 26 and 97 under 35 U.S.C. § 102(b) be reversed and dismissed.

B. Summation of Examiner's Comments and Appellants' Response.

Appellants note that in the Examiner's Answer, as in previous Office Actions, the Examiner has not even considered and addressed evidence which Appellants have submitted to prove that the presently claimed cell populations are not the same as those described in Haynesworth. Indeed, in the Answer the Examiner continues to use the same text and allegations presented in previous Office Actions without considering and addressing evidence and explanations submitted by Appellants.

The most exemplary and revealing statement (repeated yet again in the Answer[1]), is the Examiner's unsupported conclusion that, "While the prior art does not clearly disclose all of Appellants [sic] claimed limitations, ***it would appear***, that the cells claimed are a population of MSCs as disclosed by Haynesworth." *See*, Answer, page 8, 3rd paragraph (emphasis added). This conclusion is critically important because the pending rejection under 35 U.S.C. §102(b) is entirely dependent on speculation that "*it would appear*, that the cells claimed are a population of MSCs as disclosed by Haynesworth."

However, Appellants have previously addressed the Examiner's unsupported conjecture by providing manifold evidence, affidavits, and explanations establishing that Appellants' claimed cell populations are *not* the same as "a population of MSCs as disclosed by Haynesworth." Despite this, and without acknowledging and addressing Appellants' evidence submitted to the contrary, the Examiner continues to arbitrarily reject the pending claims by relying on the same speculation that "*it would appear*, that the cells claimed are a population of MSCs as disclosed by Haynesworth." In stark contrast, Appellants have

[1] *See e.g.*, Answer, page 8, 3rd paragraph ("While the prior art does not clearly disclose all of Appellants' claimed limitations, ***it would appear*** that the cells claimed are a population of MSCs as disclosed by Haynesworth"; *and,* page 10, last paragraph to page 11, first paragraph ("As such, the claimed cells ***would appear to be*** functionally the same as what the prior art calls mesenchymal stem cells differing only in the presence or absence of a few cell surface markers…").

provided internal and external evidence that Haynesworth's MSCs and the presently claimed cell populations have, at least, the following inherently different characteristics:

(i) MSCs are CD44 negative, whereas the cell populations of the present invention are CD44 positive;

(ii) MSCs are found in a *low density, platelet* containing gradient fraction, whereas the cells of the present invention are found in a *high density mononuclear* cell containing fraction;

(iii) Three specific media formulations are described as "critical" to isolation of MSCs, whereas a different media formulation is used to isolate the cells of the present invention;

(iv) MSCs have significantly reduced self-renewal capacity compared to the cell populations of the present invention (*i.e.*, a mean of 38 +/- 4 doublings for MSCs versus at least 50 population doublings for cell populations in the present application); and,

(v) MSCs acquire increasingly longer population doubling times with each passage in culture, whereas the cell populations of the present invention maintain consistent population doubling times for at least 30 to 50 population doublings.

See e.g., **Exhibit 8**: Second Kopen Declaration, pages 3-11, § B., 1-4. Accordingly, the rejection of claims 14, 21, 25, 26 and 97 under 35 U.S.C. § 102(b) is in error and Appellants respectfully request that the rejection be reversed and dismissed.

C. Detailed Comments on the Examiner's Remarks and Appellants' Response.

1. Source of Isolation of the Cells of the Invention

a. The claimed cells are not isolated in the same manner as in Haynesworth

In the Answer, the Examiner now erroneously concludes, "However, as the claimed cells are isolated *from the same source and in the same manner* as the cells of Haynesworth, the cells must necessarily be the same." *See*, Answer, page 5, last paragraph (emphasis added).

The above comment is the first time the Examiner has introduced this allegation in these proceedings. This comment also presents an example of the interminable and

piecemeal nature of examination to which the present application has been subjected.[3] Furthermore, this statement is in error for at least two reasons.

First, the record clearly shows that the cell populations of the present invention are *not* isolated "in the same manner" as the cells in Haynesworth. In fact, the record shows not only how methods described in the present application differ from those in Haynesworth (and other prior art) but also contains explanation and evidence showing *how cell populations obtained using these methods are different* from those obtained in Haynesworth's (and in other prior art publications). For example, the record shows that the manner in which the cells are initially isolated, cultured and expanded are both novel and key to generating cell populations of the invention.[4,5] More particularly, the record shows that the present application describes first obtaining an aspirate of human bone marrow and allowing cells in this heterogeneous mixture to adhere to a tissue culture-treated surface *under low oxygen conditions.* Once adherent colony forming units are produced, the cells are passaged *at low cell seeding densities.*[6,7] Thus, by isolating and passaging bone marrow-

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[3] This issue is addressed more fully by Appellants in the Conclusion section (below).

[4] *See e.g.*, Supplementary Amendment and Reply Under 37 C.F.R. § 1.111(a)(2)(B), pages 4-7 (submitted July 7, 2008) *explaining, in part, that*:

> [T]he prior art does not teach or suggest that applying a combination of low oxygen concentration and low cell density passaging to the isolation and culturing of human bone marrow cells would produce the unique population of cells that Applicants have obtained via the process recited in claim 97. In other words, obtaining the presently claimed unique, isolated population of human bone marrow cells by combining low oxygen conditions and low cell density culturing is a novel, as well as a surprising and unexpected result.
> Hence, Applicants particularly submit that no publications previously cited by the Examiner nor any prior art of which Applicants are currently aware teaches or suggests isolation of, or the possibility of isolating, the unique cell population of claim 97. Moreover, the prior art does not teach, suggest, or motivate one of skill in the art to try to obtain, or to expect that one could obtain, the presently claimed unique cell population via a combination of low oxygen condition and low cell density passaging as recited in claim 97. *See, for example*: Haynesworth *et al.*, U.S. Patent 5,733,542; Pittenger *et al.*, *Science 284*:143-147 (1999); Woodbury *et al.*, *J. Neurosci. Res. 61:* 364-370 (2000); Lee *et al.*, *Hepatology 40*: 1275-1284 (2004); Jiang *et al.*, *Nature 418*:41-49 (2002); Furcht *et al.*, U.S. Patent 7,015,037; Caplan *et al.*, U.S. Patent 5,486,359; Caplan *et al.*, U.S. Patents 5,811,094; and, Caplan *et al.*, U.S. Patent 5,837,539.

[5] *See e.g.*, Appeal Brief, page 7, penultimate paragraph.

[6] For example, as described in the specification, seeding densities of "less than about 2500 cell/cm^2, preferably less than about 1000 cells/cm^2, and most preferably less than about 100 cells/cm^2. In a particular embodiment, the initial cell density in the expansion step is between about 30 cells/cm^2 to about 50 cells/cm^2. *See*, **Exhibit 1**; Specification, page 12, lines 4-9; *see also,* Examples 1-4.

derived cells using a *combination* of *low oxygen conditions* and *low cell seeding densities*, it as been discovered that the claimed cell populations can be maintained in culture with *consistently rapid* population doubling rates, even after 30 population doublings.

In contrast to the present application, Haynesworth describes the use of a single, extremely high, cell seeding density of over 600,000 cells/cm^2 and culturing the cells isolated therein under art standard conditions comprising an "atmosphere eontaining 95% air and 5% CO_2."[8] Furthermore, Haynesworth does not mention or suggest even *the possibility* that alteration of a single factor such as cell seeding density *or* oxygen condition, *much less a combination of the two*, could determine the type of cell populations which are selected for and obtained from whole bone marrow aspirates. Accordingly, the Examiner's assertion that Appellants' cell populations are isolated "in the same manner" as cells isolated in Haynesworth is quite incorrect.

In the Answer, the Examiner also now alleges that Haynesworth teaches use of a "low oxygen" condition because Haynesworth's cells are incubated under conditions of "95% air/5% CO_2." *See*, Answer, page 5, last paragraph. This is the first time the Examiner has presented this allegation in these proceedings. This remark also presents another example of the interminable and piecemeal nature of examination to which the present application has been subjected.[9]

Furthermore, the Examiner's hindsight construction ignores the fact that culturing cells in an "atmosphere containing 95% air and 5% CO_2" (as described in Haynesworth) represents use of long-practiced, art-standard conditions and does not embody a teaching toward the use of low oxygen conditions.[10] Additionally, Haynesworth does not mention or suggest even the possibility or potential desirability of manipulating oxygen concentration in

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[7] *See also*, **Exhibit 2**: First Kopen Declaration, pages 3-4, § B (*see esp.*, Footnote 1 (summarizing isolation parameters described and used in the present application)).

[8] *See*, Haynesworth *at* col. 3, lines 55-56 and col. 4, lines 16-18 (describing seeding cells at over 600,000 cells/cm^2 wherein $50x10^6$ cells are plated per 100mm dish. [Area of 100mm (10cm) dish = $\pi \cdot R^2 = 3.14 \cdot (5\ cm)^2 = 78.5\ cm^2$; such that $50x10^6$ cells per 78.5 $cm^2 = 5.0x10^7$ cells/78.5 cm^2 or 636,943 cells/cm^2]); *and,* col. 4, lines 24-25 (describing use of an atmosphere containing 95% air and 5% CO_2).

[9] This issue is addressed more fully by Appellants in the Conclusion section (below).

[10] The 95% air and 5% CO_2 incubation conditions used in Haynesworth represent the art-standard conditions most frequently used for cultivation of mammalian cell lines. *See e.g.*, Cell Biology, *2nd Edition*, vol. 1, pages 6-11, *Ed.* J. E. Celis, Academic Press, NY (1998) (describing use of standard culture conditions "…wherein cells are cultured under atmospheric conditions (*i.e.*, 95% air) supplemented with 5% CO_2 in order to maintain a proper pH in the cell culture media (via interaction of dissolved CO_2 with $NaHCO_3$ in the culture media)).

order to effect the type of cell population obtained and derived from a heterogeneous whole bone marrow aspirate. Moreover, in addition to Haynesworth *not* teaching use of low oxygen conditions, the reference *also does not teach* use of low cell seeding densities. In fact, Haynesworth teaches away from the use of low cell seeding densities by teaching the use of a single, very high, cell seeding density.[8] Thus, the Examiner's newly rendered remark that Haynesworth allegedly teaches the isolation of cells "in the same manner" as described in the present application ignores the fact that the appealed claimed cell populations are isolated via the use of *combined* culture conditions of low oxygen *and* low cell seeding density (neither of which are taught alone, much less in combination, by Haynesworth). Thus, Haynesworth is far from representing a proper reference for use in rejecting the present claims under 35 U.S.C. § 102(b).

The Examiner also continues to overlook or ignore the fact that the two pending independent claims (14 and 97) are drawn to an isolated cell population wherein the cells maintain a population doubling rate of less than about 30 hours after 30 cell doublings. This is quite significant because, as the record amply demonstrates, Haynesworth's cell populations neither expressly nor inherently possess this characteristic, ability, or phenotype and, therefore, do not anticipate the presently claimed cell populations. *See e.g.*, **Exhibit 8**: Second Kopen Declaration, pages 8-11, § B, 4.

b. The claimed cells are isolated from a different source sub-population.

It is correct that the presently claimed cell populations and those isolated in Haynesworth are both *initially* obtained from among samples of whole bone marrow aspirates. However, whole bone marrow aspirates are a heterogeneous mixture of different cell types. In this regard, Appellants have also provided evidence that Haynesworth's MSCs are found in a different *sub-population* of whole bone marrow aspirate compared to the *sub-population* of whole bone marrow aspirate from which cell populations in the present application may be derived.

The record shows that Haynesworth's MSCs are found among *a low density, platelet-associated,* gradient fraction of cells.[11] In contrast, the gradient fractionation procedure described in Example 2 of the present application shows that Appellants' bone marrow-derived cell populations are found in a *high-density, mononuclear,* gradient fraction.[11,12]

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[11] *See*, **Exhibit 8**; Second Kopen Declaration, Section B.2, pages 4-6.

[12] *See*, **Exhibit 1**: Specification, Example 2, page 27, lines 9-19.

Moreover, as explained in the Second Kopen Declaration (**Exhibit 8**), the gradient fractionation medium used in Haynesworth (70% Percoll for isolation of low density cell types) compared to that used in the present application (HISTOPAQUE 1.119® for isolation of high density cell types), could *not* be used to isolate the same starting cell populations because of substantial differences in the separation properties of these media (*i.e.*, separation of high density versus low density cell types). *See*, **Exhibit 8**; Second Kopen Declaration, Section B.2., pages 4-5 including Exhibit B (referenced therein). Hence, bone marrow-derived cell populations isolated from these *different density gradient fractions* represent *different starting populations* of cells even before any subsequent seeding, plating, or culturing procedures are implemented. Thus, since Appellants have demonstrated that the cells in Haynesworth and cell populations of the present invention are obtained from different source sub-populations, it is erroneous to simply consider them as being the same based on an overly-simplified conclusion that they are "*from the same source.*"

Furthermore, it is also biologically and legally improper to conclude (and particularly to reject claims based on such conclusion in the face of evidence to the contrary), that both cell populations are the same just because both *originated* from bone marrow. If this same logic were applied to similar inventions, then no isolated cell population or cell line which was at some point derived from a human organism[13] would be patentable over a very first human cell population because both were derived from the same source (*i.e.*, a human). In the present case, "bone marrow" simply represents a somewhat smaller compartment in comparison to a "human" (though both comprise and give rise to many different *in vivo* and *in vitro* cell types). The Examiner's logic is, likewise, analogous to assuming that all cells derived, isolated, and propagated from a liver are the same just because they came from a liver. Yet the liver also comprises many different types of cells (*e.g.*, hepatocytes, endothelial cells, epithelial cells, smooth muscles cells, neurons, fibroblasts, immune cells, *etc.*; in addition to the various phenotypes such cells may give rise to when isolated and cultured using different *in vitro* culture conditions.)

In sum, Appellants respectfully submit that the Examiner is in error in stating that isolated cell populations of the present invention are the same as MSCs in Haynesworth based on the misleading and incorrect allegation that the cells are isolated "from the same source and in the same manner" as those in Haynesworth.

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[13] Comprising several hundred different known cell types.

2. The Cited References, Pittenger, Woodbury, and Lee, Are Not Relevant to the Claims on Appeal

On page 6, first paragraph, of the Answer the Examiner discusses the cited references, Pittenger, Woodbury, and Lee. Appellants have demonstrated and the record shows that the claimed cell populations are different and distinct from the cell populations described in each of Pittenger, Woodbury, and Lee. *See e.g.*, Appeal Brief, pages 24-27, § VII, C, 3, (b)-(d). However, these publications are actually irrelevant to the presently pending claims because, as admitted by the Examiner[14], the appealed claims are *not drawn to any of the parameters* for which the Examiner cites each reference. In particular, the pending claims are not drawn to cells that "differentiate to various mesodermal lineages" (Pittenger), are not drawn to cells that "differentiate to neurons" (Woodbury), and are not drawn to cells that "differentiate to hepatocytes" (Lee). *See*, Answer, page 6, first paragraph.

Thus, Appellants submit that none of these references, alone or combined, provide support for the Examiner's rejection of claims under 35 U.S.C. § 102(b). Furthermore, Appellants note that the Examiner's continued presentation and reliance on these irrelevant references provides an example of the interminable, cut-and-paste, *non-contemplative* nature of examination to which the present application has been repeatedly subjected.[15]

3. Limitations in the Claims on Appeal Are Not Inherent Characteristics of Prior Art Cell Populations.

The Examiner speculates that:

> Even if Appellants had identified properties of the MSCs of Haynesworth et al. that Haynesworth et al. did not or could not test for, such an identification would not render the MSCs of Haynesworth et al. patentable. As such, [Appellants'] claimed expressions and doubling rates are merely inherent characteristics of the previously known cell population.

See, Answer, page 7, penultimate paragraph. Appellants have previously shown why this statement is wrong.

As an initial matter, Appellants have not "identified properties of the MSCs of Haynesworth" because Appellants' cell populations are not the same as (*i.e.*, are different and distinct from) cell populations described in Haynesworth.

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[14] *See*, Answer, page 6, first sentence "Further, (*although unclaimed*) Appellants disclose in their specification that the cell population is pluripotent and has a particular neuronal therapeutic use" (emphasis added).

[15] This issue is addressed more fully by Appellants in the Conclusion section (below).

Second, Appellants have demonstrated and the record shows why it is wrong to contend that "the claimed expressions and doubling rates are merely inherent characteristics of the previously known cell population." In particular, Appellants have demonstrated not only differences in cell surface protein expression, but also that cell population doubling rates embodied in the claims on appeal are not inherent characteristics of Haynesworth's cells (as the Examiner alleges). For instance, Appellants have shown that Haynesworth's cells have longer population doubling times and a diminished self-renewal capacity compared to cell populations of the present invention. *See, e.g.*, Appeal Brief , pages 20-21, § VII, C, 3, (a), (i)-(ii). Moreover, the record contains evidence and testimony that population doubling times (particularly long or lengthening population doubling times) are characteristics which cannot be routinely manipulated forward and backward as the Examiner would like to believe. *See e.g.*, **Exhibit 2**: First Kopen Declaration, pages 20-23, § E, 1 through 2(A)(i)-(viii) (averring and providing evidence of the unidirectional and cumulatively irreversible nature of longer cell population doubling times). For example, Appellants note Exhibit E as discussed in **Exhibit 2** wherein a third party publication (Lennon *et al.*, *Jour. Cell. Physiol. 187*:345-355 (2001)) demonstrated that rat bone marrow mesenchymal cells cannot be switched back and forth between optimal and less optimal growth conditions and still maintain (or re-acquire) the ability to reproduce at an initial lower population doubling time. *See*, **Exhibit 2**: First Kopen Declaration, pages 21-22, § 2(A)(iv)-(v). Hence, in contrast to the Examiner's assertions, cell population doubling times are a rather inflexible, irreversible phenotypic characteristic, such that doubling times can grow *longer* with accumulated cell population doublings *in vitro*, but cannot be readily and routinely manipulated *backward* to shorter times (*i.e.*, more rapid doublings) merely by placing a slow-doubling cell population (*e.g.*, Haynesworth's cells) under new, more optimal cell culture conditions (*e.g.*, conditions used in the present application).

In a similar manner, the Examiner also broadly alleges in the Answer that "Doubling rate[,] though a property of a cell[,] is completely dependent on the culture conditions used." *See*, Answer, page 11, 2nd paragraph. As discussed above, Appellants have addressed the erroneous nature of this statement. Thus, in contrast to the Examiner's allegation, Appellants have provided substantiating evidence that cell population doubling rates are not a completely plastic phenomenon which can be whimsically changed forward and back via manipulation of culture conditions. *See e.g.*, **Exhibit 2**: First Kopen Declaration, pages 20-23, § E, 1 through 2(A)(i)-(viii). Indeed, although it is true that primary cell population doubling rates are affected by cell culture conditions, doubling rates also depend on other

factors such as, for example, whether or not initial and subsequent culture conditions have been changed or maintained (*e.g.*, oxygen conditions and/or cell seeding densities), how many doublings a cell population has undergone, type of cells being cultured (*e.g.*, liver, lung, or bone-marrow cells; differentiated or non-differentiated cells), cell genomic conditions (*e.g.*, whether or not the cells have chromosomally transformed into perpetual cell lines). *See e.g.*, **Exhibit 2**: First Kopen Declaration, pages 20-23, § E, 1 through 2(A)(i)-(viii).

Hence, the presently claimed cell populations are selected and generated as a direct result of the initial isolation and subsequent culture conditions used. However, this also means that previously isolated cell populations (such as those described in Haynesworth) cannot be made into (or, turned back into) the claimed cell populations when these same initial and subsequent culture conditions were not used from the very start. *See e.g.*, **Exhibit 2**: First Kopen Declaration, pages 21-23 § E, 2, (A), (i)-(viii). In contrast, it is precisely because of the different conditions used to initially isolate and subsequently culture the cells in Haynesworth (versus cell populations in the claims on appeal) that Haynesworth and the Appellants have isolated and produced inherently different and distinct cell populations. Furthermore, even if Haynesworth's cell populations *could* be "turned back" into the claimed cell populations, Haynesworth does not anticipate the claims on appeal under 35 U.S.C. § 102(b) because *Haynesworth never made or described, explicitly or inherently, the presently claimed cell populations*. Indeed, the application on appeal presents, for the first time, the surprising and unexpected discovery that a *combination* of low oxygen and low cell seeding density conditions permits the selection and maintenance of constant, rapid cell population doubling rates for an extensive number of cell doublings in primary populations of bone marrow-derived cells.

4. Cell Culture Density Can Have Determinative Effects on Isolated Cell Populations.

The Examiner also now categorically alleges that, "[T]he culturing of cells at a particular density does not make the cells *per se* different." This is the first time the Examiner has presented this allegation in these proceedings. As such, this remark presents yet another example of the interminable and piecemeal nature of examination to which the present application has been subjected.[16]

[16] Addressed more fully by Appellants in the Conclusion section (below).

The Examiner's comment is also biologically inaccurate. Those skilled in the art understand that cell seeding density can, indeed, make cells *per se* different. For example, Parnas & Linial have explained:

> P19 neurons in culture represent a mixed population in terms of their neurotransmitter phenotype. The cholinergic phenotype of these neurons is modulated by culture density. Cholinergic markers, such as the vesicular acetylcholine transporter, acetyl cholinesterase, and choline acetyltransferase, are expressed in about 85% of the cells in sparse cultures and are largely suppressed at high cell densities. In contrast, glutamate release is enhanced in dense P19 neuronal cultures.
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> ....
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> Cell density *in vitro* is known to affect cells in many different aspects, such as gene expression, growth, and morphology... Cell density was also implicated in regulating neuronal phenotype *in vitro*.

See, Parnas & Linial, "Culture Density Regulates Both the Cholinergic Phenotype and the Expression of the CNTF Receptor in P19 Neurons," *Jour. Molec. Neurosci., 8*:115-130 (1997) *at* abstract *and* page 116, left column, second paragraph.

As another example, Reynolds, *et al.* has taught:

> [S]tandardised conditions have been described whereby the same isolate of alveolar type II cells may be maintained *in vitro* to produce different cell cultures. Densely packed cells on ECM retained the primary cell characteristics for eight days whereas sparsely seeded cells underwent transdifferentiation, irrespective of substratum to produce cultures that were intermediate between type II and type I cells.

See, Reynolds, *et al.* "Density and Substrata are Important in Lung Type II Cell Transdifferentiation *In vitro*," *Int. Jour. Biochem. & Cell Biol., 31*:951-960 (1999) *at* page 959, left column, second paragraph.

Accordingly, it is legally and biologically improper for the Examiner to assert a rejection under 35 U.S.C. § 102(b) premised on the newly presented but erroneous assertion that "the culturing of cells at a particular density does not make the cells *per se* different."

5. *The Record Demonstrates Appellants Have Provided More Than Ample Factual Evidence.*

In view of the abundant variety of evidence that has been presented in this appeal, Appellants are stunned that the Examiner alleges, "Significantly, Appellants provide no

factual evidence whatsoever to refute the holding of anticipation or obviousness."[17] *See*, Answer, page 9, first paragraph. Indeed, in these proceedings Appellants have presented the Board, *inter alia*, with two separate affidavits including 41 pages of evidentiary testimony and 8 different exhibits of additional evidence to prove the novel and unique attributes of the presently claimed invention! *See*, Appeal Brief. Furthermore, during prosecution of this application, over a 9 year period, Appellants have had four different in-person interviews with the Examiner wherein most, if not all, of this same evidence has been discussed. *See*, **Conclusion**, § VI (below) for a more detailed summary of the prosecution history of the present application. Accordingly, an assertion that Appellants have presented no factual evidence is just plain incorrect and flies in the face of the voluminous record. Indeed, it demonstrates that Appellants have not received a thoughtful or careful examination of their claims, their arguments, or their evidence.

Appellants respectfully submit that the currently pending rejection of claims 14, 21, 25, 26 and 97 be reversed and dismissed in view of the evidence and arguments Appellants have submitted but which the Examiner has arbitrarily and capriciously ignored.

6. The Appealed Claims Contain Parameters Based on Both Cell Surface Expression Patterns and Fundamental Functional Characteristics.

On page 10, penultimate paragraph, the Examiner states "it is unclear that the presence of one or even a few differences in cell surface markers is indicative of a new cell type. This is particularly true wherein there is no apparent functional change in the cell." Similarly, the Examiner states:

> Furthermore, it is apparent from the literature on the subject that not all cell surface markers are conserved. i.e. are always present or absent from a particular cell type. As sueh, the claimed cells ***would appear*** to be functionally the same as what the prior art calls mesenchymal stem cells *differing only in the presence or absence of a few cell surface markers* that have not shown [sic] to have any bearing on the fundamental functions or characteristics of the cells.

See, Answer, page 10, last paragraph (emphasis added).

Appellants note that statements such as this represent either a misunderstanding or mischaracterization of the claims because the appealed claims incorporate parameters that differ in more than "the presence or absence of a few cell surface markers." In particular, the

[17] Appellants note the Examiner's erroneous reference to "obviousness" further evidences the cut-and-paste and non-contemplative nature of examination to which the present application has been repeatedly subjected.

appealed claims also include parameters drawn to "fundamental functions or characteristics" of the claimed cell populations. For example, the claims encompass "An isolated cell population derived from human bone marrow, wherein greater than about 91% of the cells of the cell population co-express CD49c and CD90, and *wherein the cell population maintains a doubling rate of less than about 30 hours after 30 cell doublings*." Accordingly, Appellants submit that the pending claims properly distinguish the presently claimed invention from the prior art.

7. The Examiner's Answer Contains Unsupported Subjective Conclusions and Errors Regarding The Cell Populations on Appeal.

The Examiner either does not understand or misconstrues teachings in the present application and explanations provided by Appellants. For example, *See*, Answer, page 11, penultimate paragraph to page 12, first paragraph. Appellants address each of the Examiner's comments on a point-by-point basis below.

(a) Examiner states, "Appellants have attempted to differentiate the claimed population from MSCs by indicating that the method for preparing the population differ from the methods of preparing/isolating MSCs. An analysis of Appellants' specification *does not seem to yield* a description of a method which would yield cells other than MSCs." *See*, Answer, page 11, penultimate paragraph (emphasis added).

The above quote is merely a re-worded form of the allegation that "*it would appear*, that the cells claimed are a population of MSCs as disclosed by Haynesworth..." In regard to both such statements, Appellants do indeed understand that the Examiner finds it to be an unexpected and surprising result that extraction and passaging of bone marrow-derived cells using a combination of low oxygen and low cell seeding density conditions can produce unique, isolated cell populations. However, Appellants are at a loss for understanding why the Examiner continues to maintain the same rejection, based solely on subjective conjecture and lacking any scientific, legal, or rational merit, when the grounds alleged for asserting the rejection have been rebutted via the manifold evidence and explanations submitted to the contrary (*i.e.*, Appellants' submitted evidence explaining and demonstrating the unique nature of the claimed cell populations; particularly in comparison to those described in Haynesworth). Stated otherwise, Appellants request that the final rejection of the claims be reversed because the rejections depend on the Examiner's improper and unwarranted disregard of the factual and testimonial rebuttal evidence that Appellants have supplied during the course of these proceedings.

(b) Examiner states, "Applicants argue that the claimed cells are isolated from a low density gradient fraction and that the claimed cells are isolated from a high density gradient fraction [sic]." *See*, Answer, page 11, last paragraph.

In contrast to this allegation, Appellants have never argued that the claimed cells come from *both* a low and a high density gradient fraction. If the Examiner believes this to be the case, then a specific reference to such argument is required.

On the other hand, the present application does describe and exemplify that cell populations of the invention can be isolated either with, or without, a gradient fractionation step. *Compare e.g.*, **Exhibit 1**: Specification, Examples 1 and 2; *see also e.g.*, page 10, lines 25-26 ("Alternatively, the bone marrow can be processed (e.g., fractionated by density gradient centrifugation...to derive the cell populations of the invention")). Hence, although cell populations of the invention *can be* isolated using an initial gradient fractionation step, this step is optional. Additionally, as further detailed in the next section, Appellants have explained that when gradient fractionation is used, the starting population of bone marrow-derived cells are obtained from a *high-density* cell fraction (and not a low density fraction). *See e.g.*, Appeal Brief, pages 21-22, § C, 3, (a), (iii).

(c) Examiner states, "However, as indicated in the declaration of Appellants, the media and methods for isolation are not the same and as such it is unclear how that cells can be established as different based on the density gradient fraction from which they are isolated." *See*, Answer, page 11, last paragraph.

The statement "...it is unclear how that cells can be established as different based on the density gradient fraction from which they are isolated," with all due respect, signals a lack of understanding of the function and purpose of gradient fractionation. Indeed, one of the main utilities of gradient fractionation is precisely that of separating different cell types based on differences in cell size and cell density.

All of this is on the record and was explained to the Examiner in, for example, a declaration provided in reply to a non-final office action, submitted on March 5, 2008. *See e.g.*, **Exhibit 2**: Second Kopen Declaration, pages 4-6, § B, 2; *see esp.*, Exhibit B (submitted with and discussed in **Exhibit 2**). In particular, Exhibit B is a data sheet describing Procedure No. 1119 by Sigma-Aldrich Inc. on, *inter alia*, the "Intended Use," "Procedure," and "Performance Characteristics" of HISTOPAQUE®-1119 and -1077 gradient fractionation media for use in separation of different cell types. This data sheet explains that "In 1968, Boyum described gradient density centrifugation methods for isolation of mononuclear cells from circulating blood and bone marrow." *Id.* at 1st col., 2nd paragraph.

The data sheet also explains that in using HISTOPAQUE® media "Erythrocytes should pellet to the bottom of the centrifuge tube. Granulocytes should band at the interface between the HISTOPAQUE®-1119 and the HISTOPAQUE®-1077. Mononuclear cells should band at the interface between the HISTOPAQUE®-1077 and the plasma." *Id.* at 3rd col., under "Performance Characteristics." Hence, the very purpose and function of gradient fractionation is to separate different cell types into different fractions based on their inherent differences in cell size and density. Accordingly, Appellants are perplexed by the Examiner's conclusion that it is "unclear how that cells can be established as different based on the density gradient fraction from which they are isolated" given that *separation of different cell types* based on differences in density gradient fraction *is the primary purpose* for using this technique.

The Examiner also states "However, as indicated in the declaration of Appellants, the media and methods for isolation are not the same..." *See*, Answer, page 11, last paragraph. Indeed, Appellants have *specifically pointed to the differences* in "media and methods for isolation" and the difference in "density gradient fraction" from which the claimed cell populations may be isolated, in direct comparison to the different media, methods, and gradients used for isolation of cells in Haynesworth *as additional evidence* that the claimed cell populations and Haynesworth's are not the same. In fact, *these differences standing alone* (*i.e.*, even in the absence of the additional evidence Appellants have provided) would support concluding that the presently claimed cell populations are, *at least, not likely* to be the same as Haynesworth's MSCs. Indeed, Appellants are puzzled by the Examiner's internally contradictory conclusion that since Appellants cells and Haynesworth's MSCs are isolated using ***different*** media, methods, and gradients they are, therefore, likely ***the same***!

(d) Examiner states, "It should be noted as well that the claims are not limited to a functional definition of which fraction the cells are isolated from. Claim 97 would be suggestive that the density gradient fraction is not critical. Appellants' arguments and the claims would appear to be in conflict." *See*, Answer, page 11, last paragraph to page 12, first paragraph.

The Examiner is correct, the claims are not limited, nor intended to be limited "to a functional definition of which fraction the cells are isolated from." Furthermore, Appellants' arguments and the claims are not at all in conflict because (as previously explained and as

described in the present application) density gradient fractionation is an optional step, not a required step.[18]

8. Appellants Need Not Claim All Inherent Characteristics of the Presently Claimed Cell Populations.

The Examiner also states:

> Further Appellants argue that certain marker expression profiles distinguish the claimed invention from the prior art but the expression (or lack thereof) of these markers is neither claimed nor disclosed in the specification.

See, Answer, page 12, penultimate paragraph.

The above sentence stands alone as a single paragraph in the Examiner's Answer. Appellants are perplexed as to the Examiner's intended legal implication, if any, in making this comment. Indeed, Appellants respectfully submit that this comment lacks any legal merit or consequence for the reasons explained below.

As an initial matter, the Examiner alleges the presently pending rejection of claims based on a theory of inherent anticipation under 35 U.S.C. § 102(b). Moreover, although the Examiner has not established a *prima facie* case of inherent anticipation, Appellants nonetheless elected to provide additional extrinsic evidence (such as, *inter alia*, certain marker expression profiles, which are on the record) to prove that the presently claimed cell populations are *not inherently* the same as those of Haynesworth.[19] In particular, Appellants submitted evidence demonstrating that:

(i) Haynesworth's MSCs are CD44 negative, whereas cell populations of the present invention are CD44 positive;

(ii) Haynesworth's MSCs are found in a low density, platelet containing gradient fraction, whereas cells of the present invention are found in a high density mononuclear cell containing fraction;

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[18] *See,* **Exhibit 1**: Specification, Examples 1 and 2 *and* page 10, lines 25-26 ("Alternatively, the bone marrow can be processed (e.g., fractionated by density gradient centrifugation…to derive the cell populations of the invention")); *see also*, **Exhibit 8**; Second Kopen Declaration, Section B.2, pages 4-6.

[19] In fact, even the Examiner noted that "once a proper holding of anticipation is made…the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product." *See,* Answer, page 9, first paragraph (emphasis added); citing, In re Best, 562 F.2d 1252, 1255 (CCPA 1977).

(iii) Haynesworth's MSCs require one of three specific media formulations "critical" to their isolation, whereas a different media formulation is used to isolate cells of the present invention;

(iv) Haynesworth's MSCs have reduced self-renewal capacity compared to cells of the present invention; and,

(v) Haynesworth's MSCs acquire increasingly longer population doubling times with each passage in culture, whereas cell populations of the present invention maintain consistent population doubling times for at least 30 to 50 population doublings.

See e.g., **Exhibit 8**: Second Kopen Declaration, pages 3-11, § B., 1-4. Despite these additional differences, however, the limitations in the presently pending claims are entirely sufficient for distinguishing the claimed invention from previously isolated cell populations; especially, for example, as said limitations relate to numbers (iv) and (v) above.

9. Claimed Cell Populations on Appeal Were Not Described or Produced in Haynesworth and Haynesworth's Cell Populations Cannot Be Retroactively Made Into the Claimed Cell Populations.

In a closing comment, the Examiner opines that "The majority of Appellants' arguments as summarized on page 27 of the brief are based on reported/alleged differences which have not been substantiated using the same culture conditions and, as any cell biologist of any skill in the art would know, cells react differently based on their culture conditions."

As evidenced via Appellants' previous explanations and submissions on the record, an ability of cells to "*react differently* based on their culture conditions" does not mean that Haynesworth's *in vitro* cultured eells have an infinite degree of plasticity such that they can become an isolated cell population of the presently claimed invention (as the Examiner appears to contend). *See e.g.*, **Exhibit 2**: First Kopen Declaration, pages 20-23, § D-E; *see also*, **Exhibit 8**: Second Kopen Declaration, pages 3-16, § B-G. *If* this were true, *then* it would be possible to take Haynesworth's cells and generate the claimed isolated cell populations of the present invention. However, this is not true because the eells derived from bone marrow aspirates in Haynesworth are isolated and cultured under distinctly different conditions *right from the start,* thereby giving rise to (or selecting for) a distinctly different cell population (as described in Haynesworth and in previously referenced corresponding publications). In contrast, the claimed cell populations are cultured, *from the start,* under

distinctly different conditions which critically combine low oxygen and low cell seeding densities to give rise to (or select for) human bone marrow-derived cell populations capable, for example, of maintaining a population doubling rate of less than about 30 hours even *after 30 cell doublings*. Hence, attempting to compare Haynesworth's cells to the claimed cell populations is the proverbial attempt to compare apples and oranges. Haynesworth's cell populations are inherently and demonstrably different from those presently claimed. *Id.* Indeed, it is impossible to make Haynesworth's cells become, or turn into, the claimed cell populations on appeal because to do so *one would have to use the initial and subsequent isolation and culture conditions* taught in the present application. However, *if this were done then one would not obtain Haynesworth's cells but, instead, one would have selected for and obtained the cells of Ho et al. as described in the present application*!

Moreover, even if Haynesworth's cell populations *could* be "turned back" into the presently claimed cell populations, Haynesworth does not anticipate the pending claims under 35 U.S.C. § 102(b) because *Haynesworth never made or described, explicitly or inherently, the presently claimed cell populations.*

VI. Conclusion

With all due respect, Appellants request that the currently pending rejection be reversed and that the claims on appeal be allowed to proceed to issue. *For the reasons indicated herein, Appellants particularly and respectfully request that the present application and claims* ***not*** *be remanded to the Examiner pursuant to 37 C.F.R. § 41.50 for further examination.*

Appellants are concerned and distressed by the manner in which prosecution of the present application has been unjustly delayed in the U.S. Patent and Trademark Office ("USPTO"). During prosecution of the present application, time and again, through submission of evidence and argument, Appellants have been able to distinguish their invention from each and every cited publication and reference presented (in piecemeal fashion) in one office action after another by the present Examiner. Yet, the Examiner has continued to issue rejections on repetitive and increasingly questionable grounds; as should be readily apparent from the present Examiner's Answer and Appellants' Reply. Indeed, publications cited in the most recent final Office Action[20] constitute another redundant and

[20] Exhibit 3 in the present Appeal.

cumulative round of citations referencing the same populations of bone marrow-derived cells (*i.e.*, "MSCs") over which Appellants previously distinguished their claims in response to multiple office actions. Further in this regard, Appellants note that since September 21, 2001 (*9 years*) the present application has been subjected to:

- *Four rounds of non-final rejections and responses;*
- *Two rounds of final rejections and responses;*
- *One request for continued examination;*
- *One notice of allowance issued;*
- *One issue fee payment presented;*
- *One USPTO-initiated withdrawal from issue;*
- *One lengthy, USPTO-mandated suspension of prosecution for a potential interference that was never declared; and,*
- *Four in-person interviews with the Examiner; including two with the Examiner's Supervisor.*

In view of Appellants' submissions on the record and arguments on this appeal, it is respectfully submitted that the presently pending claims are in condition for prompt and immediate allowance. It is Appellants' belief that only careful consideration by this Board will finally give them their "day in court." Appellants urge the Board to, once and for all, end the interminable and, frankly, hopeless proceedings before the Examiner.

Appellants respectfully request and urge the Board to finally pass this case to the issuance it rightfully deserves.

Respectfully submitted,

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